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(54) **MACROCYCLIC AMINOPHOSPHONIC ACID COMPLEXES, THEIR PREPARATION, FORMULATIONS AND USE.**

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## D scripti n

The present invention concerns macrocyclic aminophosphonic acid complexes for the treatment of cancer, especially the treatment of calcific tumors and for the relief of bone pain, the method of treatment of calcific tumors, and compositions and formulations having as their active ingredient a radionuclide complexed with a macrocyclic aminophosphonic acid, and the process for preparing the macrocyclic aminophosphonic acid complexes.

The development of bone metastasis is a common and often catastrophic event for a cancer patient. The pain, pathological fractures, frequent neurological deficits and forced immobility caused by these metastatic lesions significantly decrease the quality of life for the cancer patient. The number of patients that contract metastatic disease is large since nearly 50 percent of all patients who contract breast, lung or prostate carcinoma will eventually develop bone metastasis. Bone metastasis are also seen in patients with carcinoma of the kidney, thyroid, bladder, cervix and other tumors, but collectively, these represent less than 20 percent of patients who develop bone metastasis. Metastatic bone cancer is rarely life threatening and occasionally patients live for years following the discovery of the bone lesions. Initially, treatment goals center on relieving pain, thus reducing requirements for narcotic medication and increasing ambulation. Clearly, it is hoped that some of the cancers can be cured.

The use of radionuclides for treatment of cancer metastatic to the bone dates back to the early 1950's. It has been proposed to inject a radioactive particle-emitting nuclide in a suitable form for the treatment of calcific lesions. It is desirable that such nuclides be concentrated in the area of the bone lesion with minimal amounts reaching the soft tissue and normal bone. Radioactive phosphorus (P-32 and P-33) compounds have been proposed, but the nuclear and biolocalization properties limit the use of these compounds. [See for example, Kaplan, E., et al., *Journal of Nuclear Medicine* 1(1), 1 (1960) and U.S. Patent 3,965,254.]

Another attempt to treat bone cancer has been made using phosphorus compounds containing a boron residue. The compounds were injected into the body (intravenously) and accumulated in the skeletal system. The treatment area was then irradiated with neutrons in order to activate the boron and give a therapeutic radiation dose. (See U.S. Patent 4,399,817).

The use of radionuclides for calcific tumor therapy is discussed in published European patent application 176,288 where the use of Sm-153, Gd-159, Ho-166, Lu-177 or Yb-175 complexed with certain ligands selected from ethylenediaminetetraacetic acid (EDTA) or hydroxyethylethylenediaminetriacetic acid (HEEDTA) is disclosed.

In the above mentioned procedures, it is not possible to give therapeutic doses to the tumor without substantial damage to normal tissues. In many cases, especially for metastatic bone lesions, the tumor has spread throughout the skeletal system and amputation or external beam irradiation is not practical. (See *Seminars in Nuclear Medicine*, Vol. IX, No. 2, April, 1979).

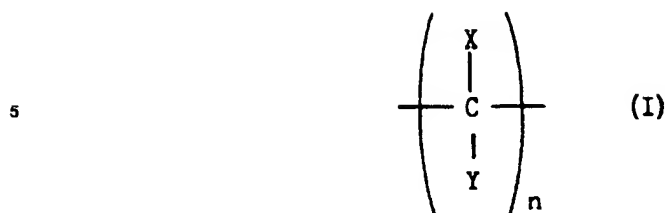
The use of Re-186 complexed with a diphosphonate has also been proposed. [Mathieu, L. et al., *Int. J. Applied Rad. & Isotopes* 30, 725-727 (1979); Weinenger, J., Ketrang, A. R., et al., *Journal of Nuclear Medicine* 24(5), 125 (1983)]. However, the preparation and purification needed for this complex limits its utility and wide application.

Strontium-89 has also been proposed for patients with metastatic bone lesions. However, the long half-life (50.4 days), high blood levels and low lesion to normal bone ratios limit the utility. [See Firusian, N., Mellin, P., Schmidt, C. G., *The Journal of Urology* 116, 764 (1976); Schmidt, C. G., Firusian, N., *Int. J. Clin. Pharmacol.* 93, 199-205, (1974).]

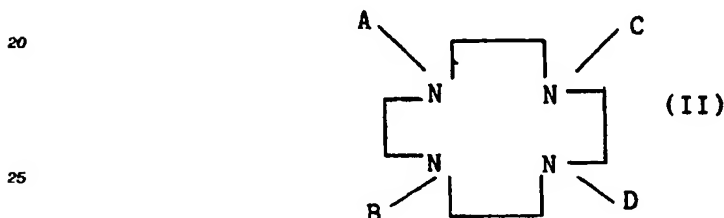
A palliative treatment of bone metastasis has been reported which employed I-131 labeled  $\alpha$ -amino-(3-iodo-4-hydroxybenzylidene)diphosphonate [Eisenhut, M., *Journal of Nuclear Medicine* 25(12), 1358-1361 (1984)]. The use of radioactive iodine as a therapeutic radionuclide is less than desirable due to the well known tendency of iodine to localise in the thyroid. Eisenhut lists iodide as one of the possible metabolites of this compound.

Surprisingly, the present invention overcomes many of the above noted problems. The present invention concerns at least one composition having a radionuclide complexed with a macrocyclic aminophosphonic acid, such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or its physiologically acceptable salt, which composition causes minimal damage to normal tissue when administered in the method of the invention. Surprisingly, the present complex is more effective at a lower ligand to metal molar ratio than has been known previously in the art.

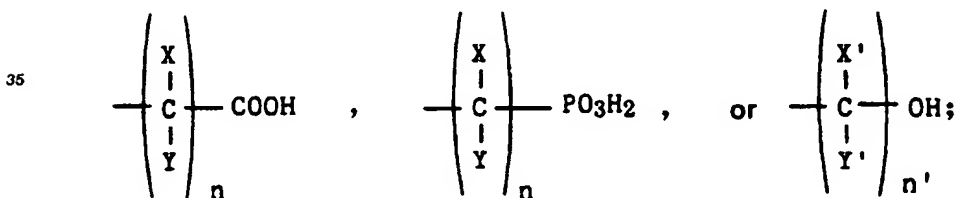
Particularly, this invention concerns a composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorous are interconnected by an alkylene or substituted alkylene radical of the formula



10 wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the proviso that when  $n > 1$ , each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, and  
 15 wherein the resulting composition is therapeutically effective. Particularly preferred are macrocyclic moieties of Formula (I) where X and Y are hydrogen and n is 1. Especially preferred are a certain macrocyclic aminophosphonic acid of the structure



30 wherein: substituents A, B, C and D are independently hydrogen, hydrocarbon radicals having from 1-8 carbon atoms, or a moiety of the formula



and physiologically acceptable salts of the acid radicals, wherein: X, Y and n are as defined before; X' and Y' are independently hydrogen, methyl or ethyl radicals; n' is 2 or 3, with the proviso that at least two of said nitrogen substituents is a phosphorus-containing group. The preferred macrocyclic aminophosphonic acid is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid (DOTMP). The composition  
 45 can be administered as a formulation with suitable pharmaceutically acceptable carriers. The present invention includes the the complex, composition or formulation described herein for use in combination with one or more other agents, drugs, treatments and/or radiation sources which assist in therapy of calcific tumors or relief of bone pain .

50 Certain compositions containing these complexes have been found useful for therapy of calcific tumors in animals. The administration of the therapeutic compositions can be palliative to the animal, for example by alleviating pain and/or inhibiting tumor growth and/or causing regression of tumors and/or destroying the tumors. As will be more fully discussed later, the properties of the radionuclide, of the macrocyclic aminophosphonic acid and of the complex formed therefrom are important considerations in determining  
 55 the effectiveness of any particular composition employed for such treatment.

In addition, the present invention also includes formulations having at least one of the radionuclide(s) complexed with at least one of the macrocyclic aminophosphonic acids as defined above , especially those macrocyclic aminophosphonic acids of Formula (II), and a pharmaceutically acceptable carrier, xipient or

vehicle thereof. The methods for preparing such formulations are well known. The formulations are sterile and may be in the form of a suspension, injectable solution or other suitable pharmaceutically acceptable formulations. Pharmaceutically acceptable suspending media, with or without adjuvants, may be used. The sterile compositions are suitable for administration to an animal wherein the composition is defined as before and has the radionuclide in dosage form present in an amount containing at least 0.02 mCi per kilogram of body weight of said animal, preferably at least 0.2 mCi per kilogram of body weight of said animal.

Particle-emitting radionuclides employed in the compositions of the invention are capable of delivering a high enough localized ionisation density to alleviate pain and/or inhibit tumor growth and/or cause regression of tumors, and/or destroy the tumor and are capable of forming complexes with the macrocyclic aminophosphonic acid ligands described herein. The radionuclides found to be useful in the practice of the invention are Samarium-153 (Sm-153), Holmium-166 (Ho-166), Ytterbium-175 (Yb-175), Lutetium-177 (Lu-177), Yttrium-90 (Y-90) and Gadolinium-159 (Gd-159).

For the purpose of convenience, the compositions having a radionuclide-macrocyclic aminophosphonic acid complex of the present invention will frequently be referred to herein as "radionuclide compositions" or "compositions" and the macrocyclic aminophosphonic acid derivative referred to as the "ligand" or "chelant".

As used herein, the term "animals" means warm blooded mammals, including humans, and is meant to encompass animals in need of treatment for calcific tumors or in need of relief of bone pain.

The term "calcific tumors" includes primary tumors, where the skeletal system is the first site of involvement, invasive tumors where the primary tumor invades the skeletal system or other tissue tumors which calcify, and metastatic bone cancer where the neoplasm spreads from other primary sites, e.g. prostate and breast, into the skeletal system.

For the purpose of the present invention, the complexes described herein and physiologically acceptable salts thereof are considered equivalent in the therapeutically effective compositions. Physiologically acceptable salts refer to the acid addition salts of those bases which will form a salt with at least one acid group of the ligand or ligands employed and which will not cause a significant adverse physiological effect when administered to an animal at dosages consistent with good pharmacological practice; some examples of such practice are described herein. Suitable bases include, for example, the alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate and the like, ammonia, primary, secondary and tertiary amines and the like. Physiologically acceptable salts may be prepared by treating the macrocyclic aminophosphonic acid as defined above, especially those of Formula (II), with an appropriate base.

The formulations of the present invention are in the solid or liquid form containing the active radionuclide complexed with the ligand. These formulations may be in kit form such that the two components are mixed at the appropriate time prior to use. Whether premixed or as a kit, the formulations usually require a pharmaceutically acceptable carrier. Additionally, for stability and other factors, if the formulations are complexed with the radionuclide prior to shipment to the ultimate user, the formulation having the complex and a buffer present are frozen in a kit form, and which frozen formulation is later thawed prior to use.

Injectable compositions of the present invention may be either in suspension or solution form. In the preparation of suitable formulations it will be recognized that, in general, the water solubility of the salt is greater than the free acid. In solution form the complex (or when desired the separate components) is dissolved in a pharmaceutically acceptable carrier. Such carriers comprise a suitable solvent, preservatives such as benzyl alcohol, if needed, and buffers. Useful solvents include, for example, water, aqueous alcohols, glycols, and phosphonate or carbonate esters. Such aqueous solutions contain no more than 50 percent of the organic solvent by volume.

Injectable suspensions as compositions of the present invention require a liquid suspending medium, with or without adjuvants, as a carrier. The suspending medium can be, for example, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous carboxymethylcellulose. Suitable physiologically acceptable adjuvants, if necessary to keep the complex in suspension, may be chosen from among thickeners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many surfactants are also useful as suspending agents, for example, lecithin, alkylphenol, polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters. Many substances which affect the hydrophobicity, density, and surface tension of the liquid suspension medium can assist in making injectable suspensions in individual cases. For example, silicone antifoams, sorbitol, and sugars are all useful suspending agents.

Complexes employed in the compositions or formulations of the present invention must fit certain criteria insofar as possible as discussed below.

One criteria concerns the selection of the radionuclide. While the properties of the radionuclide are important, the overall properties of the composition containing the radionuclidemacrocylic aminophosphonic acid complex is the determining factor. The disadvantages of any one property may be overcome by the superiority of one or more of the properties of either ligand or radionuclide and their combination, as employed in the composition must be considered in toto.

There is a need for compositions possessing the following criteria by which it is possible to deliver therapeutic radiation doses to calcific tumors with minimal doses to soft tissue. For example, the radionuclide must be delivered preferentially to the bone rather than to soft tissue. Most particularly, uptake of the radionuclide in either liver or blood is undesirable. Additionally, the radionuclide should be cleared rapidly from non-osseous tissue to avoid unnecessary damage to such tissues, e.g., it should clear rapidly from the blood.

The proposed use for the compositions and formulations of this invention is the therapeutic treatment of calcific tumors in animals. As used herein, the term "calcific tumors" includes primary tumors where the skeletal system is the first site of involvement, or other tissue tumors which calcify, or metastatic bone cancer where the neoplasm spreads from other primary sites, such as prostate and breast, into the skeletal system. This invention provides a means of alleviating pain and/or reducing the size of, and/or inhibiting the growth and/or spread of, or causing regression of and/or destroying the calcific tumors by delivering a therapeutic radiation dose.

The composition or formulation may be administered as a single dose or as multiple doses over a longer period of time. Delivery of the radionuclide to the tumor must be in sufficient amounts to provide the benefits referred to above.

The "effective amount" or "therapeutically effective amount" of radionuclide composition to be administered to treat calcific tumors will vary according to factors such as the age, weight and health of the patient, the calcific tumor being treated, the treatment regimen selected as well as the nature of the particular radionuclide composition to be administered. For example, less activity will be needed for radionuclides with longer half lives. The energy of the emissions will also be a factor in determining the amount of activity necessary. The compositions of this invention may also be employed at doses which are useful but not therapeutic.

A suitable dose of the composition or formulation of this invention for use in this invention is at least about 0.02 mCi per Kg of body weight. A "therapeutically effective dose" of the composition or formulation of this invention for use in this invention is at least about 0.2 mCi per Kg of body weight.

The effective amount used to treat calcific tumors will typically be administered, generally by administration into the bloodstream, in a single dose or multipule doses. The amounts to be administered to achieve such treatment are readily determined by one skilled in the art employing standard procedures.

The radionuclide and ligand may be combined under any conditions which allow the two to form a complex. Generally, mixing in water at a controlled pH (the choice of pH is dependent upon the choice of ligand and radionuclide) is all that is required. The complex formed is by a chemical bond and results in a relatively stable radionuclide composition, e.g. stable to the disassociation of the radionuclide from the ligand.

The macrocylic aminophosphonic acid complexes when administered at a ligand to metal molar ratio of at least about 1:1, preferably from 1:1 to 3:1, more preferably from 1:1 to 1.5:1, give biodistributions that are consistent with excellent skeletal agents. By contrast, certain other aminophosphonic acid complexes result in some localization in soft tissue (e.g. liver) if excess amounts of ligand are not used. A large excess of ligand is undesirable since uncomplexed ligand may be toxic to the patient or may result in cardiac arrest or hypocalcemic convulsions. In addition, the macrocylic aminophosphonic acid ligands are useful when large amounts of metal are required (i.e. for metals that have a low specific activity). In this case, the macrocylic aminophosphonic acid ligands have the ability to deposit larger amounts of activity in the bone than is possible when using non-cyclic aminophosphonic acid ligands.

A preferred embodiment of the present invention is a therapeutically effective composition or formulation containing complexes of at least one radionuclide of Gd-159, Ho-166, Lu-177, Sm-153, Y-90 and Yb-175 with DOTMP or a physiologically acceptable salt(s) thereof.

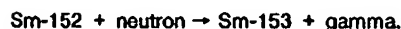
Combinations of the various above noted radionuclides can be administered for the therapeutic treatment of calcific tumors. The combinations can be complexed as herein described by complexing them simultaneously, mixing two separately complexed radionuclides, or administering two different complexed radionuclides sequentially. It may be possible to achieve the same beneficial results of high delivery of the radionuclide to the area of the tumor, but with little soft tissue damage, by administering the ligand and the

radionuclide in a manner which allows formation of the radionuclidechelant complex *in situ* such as by simultaneous or near simultaneous administration of the radionuclide and an appropriate amount of ligand or by the administration of ligand and a radionuclide complexed with a weaker ligand, i.e., one which undergoes ligand exchange with the ligands of this invention, such that the desired radionuclide-chelant complex is formed via ligand exchange *in situ*. The composition or formulation may be administered as a single dose or as multiple doses over a longer period of time.

Aminophosphonic acids can be prepared by a number of known synthetic techniques. Of particular importance is the reaction of a compound containing at least one reactive amine hydrogen with a carbonyl compound (aldehyde or ketone) and phosphorous acid or derivative thereof. The amine precursor (1,4,7,10-tetraazacyclododecane) employed in making the macrocyclic aminophosphonic acids is a commercially available material.

Methods for carboxyalkylating to give amine derivatives containing a carboxyalkyl group are well known (U.S. 3,726,912) as are the methods which give alkyl phosphonic and hydroxyalkyl (U.S. 3,398,198) substituents on the amine nitrogens.

Radionuclides can be produced in several ways. In a nuclear reactor, a nuclide is bombarded with neutrons to obtain a radionuclide, e.g.



Another process for obtaining radionuclides is by bombarding nuclides with linear accelerator or cyclotron-produced particles. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures. The process for obtaining the radionuclide is not critical to the present invention.

For example, to irradiate  $\text{Sm}_2\text{O}_3$  for production of Sm-153, the desired amount of target was first weighed into a quartz vial, the vial was flame sealed under vacuum and welded into an aluminum can. The can was irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial was removed and transferred to a glove box, crushed into a glass vial which was then sealed with a rubber septum and an aluminum crimp cap. One milliliter of 1 to 4M HCl was then added to the vial via syringe to dissolve the  $\text{Sm}_2\text{O}_3$ . Once dissolved, the solution was diluted to the appropriate volume by addition of water. The solution was removed from the original dissolution vial which contains shards of the crushed quartz vial and transferred via syringe to a clean glass serum vial. This solution was then used for complex preparation. Similar procedures can be used to prepare Lu-177, Yb-175, Gd-159, Y-90 and Ho-166.

The invention described herein provides a means of delivering a therapeutic amount of radioactivity to calcific tumors. However, it may also be desirable to administer a "sub-therapeutic" amount (i.e. "useful amount") to determine the fate of the radionuclide using a scintillation camera prior to administering a therapeutic dose. Therapeutic doses will be administered in sufficient amounts to alleviate pain and/or inhibit tumor growth and/or cause regression of tumors and/or kill the tumor. Amounts of radionuclide needed to provide the desired therapeutic dose will be determined experimentally and optimized for each particular composition. The amount of radioactivity required to deliver a therapeutic dose will vary with the individual composition employed. For example, less activity will be needed for radionuclides with longer half-lives. The energy of the emissions will also be a factor in determining the amount of activity necessary. The composition to be administered may be given in a single treatment or fractionated into several portions and administered at different times. Administering the composition in fractionated doses may make it possible to minimize damage to non-target tissue. Such multiple dose administration may be more effective.

The compositions of the present invention may be used in conjunction with other active agents and/or ingredients that enhance the therapeutic effectiveness of the compositions and/or facilitate easier administration of the compositions.

Studies to determine the qualitative biodistribution of the various radionuclides were conducted by injecting the compositions into rats and obtaining the gamma ray images of the entire animal at various times up to two hours after injection.

Quantitative biodistributions were obtained by injecting 50-100 microliters of the composition into the tail vein of unanesthetized male Sprague Dawley rats. The rats were then placed in cages lined with absorbent paper in order to collect all urine excreted prior to sacrifice. After a given period of time, the rats were sacrificed by cervical dislocation and the various tissues dissected. The samples were then rinsed with saline, blotted dry on absorbent paper and weighed. The radioactivity in the samples was measured with a NaI scintillation counter.

The following examples are included to aid in the understanding of the invention but are not to be construed as limiting the invention.

## Preparation of Starting Materials

Example A: Preparation of DOTMP

5 In a 100-mL three necked round-bottomed flask equipped with a thermometer, reflux condenser, and heating mantle was added 3.48 g (20.2 mmole) of 1,4,7,10-tetraazacyclododecane and 14 ml of water. This solution was treated with 17.2 mL of concentrated HCl and 7.2 g of  $\text{H}_3\text{PO}_3$  (87.8 mmole) and heated to 105 °C. The refluxing suspension was stirred vigorously and treated dropwise with 13 g (160.2 mmole) of formaldehyde (37 wt percent in water) over a one hour period. At the end of this time the reaction was  
 10 heated at reflux an additional 2 hours after which the heat was removed and the reaction solution allowed to cool and set at room temperature for 62.5 hours. The reaction solution was then concentrated *in vacuo* at 40 °C to a viscous reddish brown semisolid. A 30 mL portion of water was added to the semisolid which started to dissolve but then began to solidify. The whole suspension was then poured into 400 mL of acetone with vigorously stirring. The resulting off-white precipitate was vacuum filtered and dried overnight  
 15 to give 10.69 g (97 percent yield) of crude DOTMP. A 2.0 g (3.65 mmole) sample of the crude DOTMP was dissolved in 2 mL of water by the addition of 700  $\mu\text{L}$  of concentrated ammonium hydroxide (10.0 mmole) in 100  $\mu\text{L}$  portions to give a solution at pH of 2-3. This solution was then added all at once to 4.5 mL of 3N HCl (13.5 mmole), mixed well, and allowed to set. Within one hour small squarish crystals had begun to form on the sides of the glass below the surface of the liquid. The crystal growth was allowed to continue  
 20 undisturbed for an additional 111 hours after which time the crystals were gently bumped off of the vessel walls, filtered, washed with 3 mL portions of water, four times, and air dried to constant weight to give 1.19 g (60 percent yield) of white crystalline solid DOTMP.

Example B: Preparation of DOTMP

25 A 250 mL three-necked, round-bottomed flask was loaded with 6.96 g (0.04 moles) of 1,4,7,10-tetraazacyclododecane. To this flask was added 14.5 g (0.177 moles) of phosphorous acid, 30 mL of deionized water and 28 mL of concentrated hydrochloric acid (0.336 moles).

The flask was attached to a reflux condenser and fitted with a stir bar, and a thermometer adapted with a thermowatch controller. A separate solution of 26.0 g (0.32 moles) of aqueous 37 percent formaldehyde solution was added to a 100 mL addition funnel and attached to the flask. The flask was brought to reflux temperature (about 105 °C) with vigorous stirring. The formaldehyde solution was added dropwise over a 30-40 minute interval. The solution was heated and stirred for an additional three hours then cooled slowly to ambient temperature.

35 The reaction solution was transferred to a 500 mL round-bottomed flask and attached to a rotary evaporation apparatus. The solution was taken down to a viscous, amber semi-solid (note - temperature never exceeded 40 °C). This semi-solid was treated with approximately 300 mL of HPLC grade acetone producing a light brown, sticky viscous oil. This oil was dissolved in 22 mL of water and added slowly with vigorous stirring to 1L of acetone. The acetone was decanted and the light colored oil dried under vacuum  
 40 to give 16.6 g (76 percent yield) of crude DOTMP. To 13.1 g of this crude DOTMP was added 39.3 g of deionized water along with a seed crystal and the solution allowed to stand overnight. The resulting precipitate was vacuum filtered, washed with cold water, and dried under vacuum to give 4.75 g of DOTMP (36 percent yield).

A further purification was performed by dissolving 3.0 g (5.47 mmole) of DOTMP from above in 3 mL of  
 45 water by the addition of 2.2 mL (31.5 mmole) of concentrated ammonium hydroxide. This solution was made acidic by the addition of 2.4 mL (28.8 mmole) of concentrated HCl at which time a white solid precipitated. This precipitate was vacuum filtered and dried to give 2.42 g (81 percent yield) of purified DOTMP characterized by a singlet at 11.5 ppm (relative to 85 percent  $\text{H}_3\text{PO}_4$ ) in the  $^{31}\text{P}$  decoupled NMR spectrum.

Example C: Preparation of Sm-153

Sm-153 can be produced in a reactor such as the University of Missouri Research Reactor. Sm-153 is produced by irradiating 99.06 percent enriched  $^{152}\text{Sm}_2\text{O}_3$  in the first row reflector at a neutron flux of  $8 \times 10^{13}$  neutron/cm<sup>2</sup>•sec. Irradiations were generally carried out for 50 to 60 hours, yielding a Sm-153 specific activity of 1000-1300 Ci/g.  
 55

To irradiate  $\text{Sm}_2\text{O}_3$  for production of Sm-153, the desired amount of target is first weighed into a quartz vial, the vial flame sealed under vacuum and welded into an aluminum can. The can is irradiated for the



desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial is removed and transferred to a glove box, opened into a glass vial which is then sealed. An appropriate amount of a solution of hydrochloric acid is then added to the vial via syringe in order to dissolve the  $\text{Sm}_2\text{O}_3$ . Once the  $\text{Sm}_2\text{O}_3$  is dissolved, the Samarium solution is diluted to the appropriate volume by addition of water. The solution is removed from the original dissolution vial which contains the chards of the quartz irradiation vial, and transferred via syringe to a clean glass serum vial.

#### Example D: Preparation of Ho-166

Holmium-166 is prepared by weighing 0.5-1.0 mg of  $\text{Ho}_2\text{O}_3$  into a quartz vial. The vial is sealed and placed in an aluminum can which is welded shut. The sample is irradiated (usually for about 24-72 hours) in the reactor (first row reflector, neutron flux of  $8 \times 10^{13}$  neutron/cm<sup>2</sup>·sec). After irradiation, the vial is opened and the oxide is dissolved using 4N HCl. Heating may be necessary. Water is then used to dilute the sample to an appropriate volume.

#### Example E: Preparation of Gd-159

Gadolinium-159 is prepared by sealing gadolinium oxide (1.1 mg) in a quartz vial. The vial is welded inside an aluminum can and irradiated for 30 hours in a reactor at a neutron flux of  $8 \times 10^{13}$  neutron/cm<sup>2</sup>·sec. The contents of the quartz vial is dissolved using HCl. Water is added to obtain a solution of Gd-159 in 0.1N HCl.

#### Example F: Preparation of Y-90

A non-radioactive Ytterium (Y) solution was prepared by dissolving 15.1 mg of  $\text{YCl}_3 \cdot 6\text{H}_2\text{O}$  in 11.24 mL of water. A quantity of 1500  $\mu\text{L}$  of this solution was added to a vial containing 0.5 mL of Y-90 solution (prepared by neutron irradiation of 1 mg of  $\text{Y}_2\text{O}_3$  followed by dissolution in 1N HCl to give a final volume of 0.5 mL).

#### Example G: Preparation of Yb-175 and Lu-177

When the procedure of Examples C, D, E or F are repeated using the appropriate oxide, the radioisotopes of Ytterbium-175 (Yb-175) and Lutetium-177 (Lu-177) are prepared.

#### Preparation of Final Products

#### Example 1: Preparation and Biodistribution of Sm-DOTMP and Sm-153-DOTMP

The ligand of Example A (22 mg) was dissolved in 878  $\mu\text{L}$  of distilled water and 15  $\mu\text{L}$  of 50 percent NaOH. A volume of 15  $\mu\text{L}$  of this solution was transferred to a vial containing 1.5 mL of Sm solution (0.3 mM Sm in 0.1N HCl spiked with 2  $\mu\text{L}$  of Sm-153 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Sm found as a complex was >99 percent as determined by ion exchange chromatography. This yielded a solution containing Sm at 0.3 mM with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100  $\mu\text{L}$  of the Sm solution described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100  $\mu\text{L}$  standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table I. The numbers represent the average of 3 rats per data point.

TABLE I

% INJECTED DOSE IN SEVERAL TISSUES FOR Sm-DOTMP <sup>1</sup>	
Tissue	% Dose
Bone	58.1
Liver	0.06
Kidney	0.27
Spleen	0.004
Muscle	0.15
Blood	0.004

<sup>1</sup>Ligand to Sm Molar Ratio of approximately 1.5

#### Example 2: Preparation and Biodistribution of Ho-DOTMP and Ho-166-DOTMP

The ligand of Example A (22 mg) was dissolved in 878  $\mu$ L of distilled water and 15  $\mu$ L of 50 percent NaOH. A volume of 30  $\mu$ L of this solution was transferred to a vial containing 1.5 mL of Ho solution (0.6 mM Ho in 0.1N HCl spiked with 2  $\mu$ L of Ho-166 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Ho found as a complex was greater than 99 percent as determined by ion exchange chromatography. This yielded a solution containing 0.6 mM Ho with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100  $\mu$ L of the Ho solution described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100  $\mu$ L standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table II. The numbers represent the average of 3 rats per data point.

TABLE II

% INJECTED DOSE IN SEVERAL TISSUES FOR Ho-DOTMP <sup>1</sup>	
Tissue	% Dose
Bone	57
Liver	0.07
Kidney	0.4
Spleen	0.006
Muscle	0.3
Blood	0.07

<sup>1</sup> Ligand to Ho Molar Ratio of approximately 1.5

#### Example 3: Preparation and Biodistribution of Sm-DOTMP, Sm-153-DOTMP, Ho-DOTMP and Ho-166-DOTMP

A quantity of 14.5 mg of the ligand of Example B was placed in a vial and dissolved in 760  $\mu$ L of water and 5  $\mu$ L of 50 percent NaOH. A volume of 1100  $\mu$ L of Sm solution (0.3 mM Sm in 0.1N HCl) which was spiked with Sm-153, was placed in a separate vial and 10  $\mu$ L of the ligand solution was added. The pH of

the solution was adjusted to 7-8 using NaOH and the solution was passed through 3 plastic columns containing 1.5 mL of cation exchange resin (Sephadex™ C-25 from Pharmacia). The amount of Sm as a complex was determined to be 99 percent by cation exchange chromatography.

5 A volume of 1100 µL of Ho solution (0.6 mM Ho in 0.1N HCl) which was spiked with Ho-166, was placed in a separate vial and 20 µL of the above ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the solution was passed through 2 plastic columns containing 1.5 mL of cation exchange resin (Sephadex C-25 from Pharmacia). The amount of Ho as a complex was determined to be 99 percent by cation exchange chromatography.

10 Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table III. The numbers represent the average of 3 rats per data point.

TABLE III

20	% INJECTED DOSE IN SEVERAL TISSUES FOR DOTMP METAL COMPLEXES		
	Tissue	Sm	Ho
	Bone	50	64
	Liver	0.37	0.19
25	Kidney	0.29	0.32
	Spleen	0.04	0.05
	Muscle	0.49	0.22
30	Blood	0.12	0.17

#### Example 4: Preparation and Biodistribution of Gd-DOTMP and Gd-159-DOTMP

35 The ligand of Example B (14.5 mg) was placed in a vial and dissolved in 760 µL of water and 5 µL of 50 percent NaOH. A volume of 1000 µL of Gd solution (0.3 mM Gd in 0.1N HCl) which contained tracer quantities of Gd-159, was placed in a separate vial and 15 µL of the ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the amount of Gd as a complex was determined to be >99 percent by cation exchange chromatography.

40 A Sprague Dawley rat was allowed to acclimate for five days then injected with 175 µL of the solution described above via a tail vein. The rat weighed 155 g at the time of injection. After 2 hours the rat was killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 175 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table IV.

50

55

TABLE IV

% INJECTED DOSE IN SEVERAL TISSUES FOR Gd-DOTMP <sup>1</sup>	
Tissue	% Dose
Bone	50
Liver	0.08
Kidney	0.25
Spleen	None Detected*
Muscle	0.08
Blood	0.06

<sup>1</sup> Ligand to Gd molar ratio of approximately 1.5

\* counts in the spleen were below background background

#### 20 Example 5: Preparation and Biodistribution of Lu-DOTMP and Lu-177-DOTMP

The ligand of Example B (15.8 mg) was dissolved in 963  $\mu$ L of distilled water and 8  $\mu$ L of 50 percent NaOH. A volume of 15  $\mu$ L of this solution was transferred to a vial containing 1.5 mL of Lu solution (0.3 mM Lu in 0.1N HCl spiked with 2  $\mu$ L of Lu-177 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Lu found as a complex was >99 percent by ion exchange chromatography. This yielded a solution containing 0.3 mM Lu with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100  $\mu$ L of the Lu solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100  $\mu$ L standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table V. The numbers represent the average of 3 rats per data point.

TABLE V

% INJECTED DOSE IN SEVERAL TISSUES FOR Lu-DOTMP <sup>1</sup>	
Tissue	% Dose
Bone	54
Liver	0.08
Kidney	0.3
Spleen	0.006
Muscle	0.04
Blood	0.09

<sup>1</sup> Ligand to Lu molar ratio of approximately 1.5

#### Example 6: Preparation and Biodistribution of Y-DOTMP and Y-90-DOTMP

To the solution of Y and Y-90 prepared in Example F was added 200  $\mu$ L (0.0266 moles) of DOTMP from Example B in water and the pH of the solution adjusted to 7.5 using 50 percent NaOH and 1N NaOH. The percent of the Y as a complex was determined by cation exchange chromatography to be >99 percent. This yielded a solution with a ligand to metal molar ratio of approximately 1.7.

Sprague Dawley rats were allowed to acclimate for eight days then injected with 150  $\mu\text{L}$  of the Y solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 150  $\mu\text{L}$  standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VI. The numbers represent the average of 5 rats per data point.

TABLE VI

% INJECTED DOSE IN SEVERAL TISSUES FOR Y-DOTMP <sup>1</sup>	
Tissue	% Dose
Bone	33
Liver	0.06
Kidney	0.35
Spleen	0.01
Muscle	0.31
Blood	0.12

<sup>1</sup> Ligand to Y molar ratio of approximately 1.7

#### Example W (Comparative)

To a vial containing 0.5 mL of Y-90 solution (prepared by the irradiation of 1 mg of  $\text{Y}_2\text{O}_3$  followed by dissolution in 1.1N HCl to give a final volume of 0.5 mL) was added 1.5 mL of water to give a  $8.86 \times 10^{-3}$  molar solution of Y containing tracer Y-90. To 2 mL ( $1.772 \times 10^{-5}$  mole) of this solution was added 133  $\mu\text{L}$  ( $1.676 \times 10^{-4}$  mole) of 1.26M ethylenediaminetetramethylenephosphonic acid (EDTMP) solution where upon the solution became turbid. The solution cleared up upon addition of 50  $\mu\text{L}$  of 50 percent NaOH. To this solution was added 40  $\mu\text{L}$  ( $5.04 \times 10^{-5}$  mole) more of 1.26M EDTMP solution. The pH of the resulting solution was 7.5 and the percent of the Y as a complex was determined by cation exchange chromatography to be >99 percent. This yielded a solution with a ligand to metal molar ratio of approximately 123.

Sprague Dawley rats were allowed to acclimate for eight days then injected with 150  $\mu\text{L}$  of the Y solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 150  $\mu\text{L}$  standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table W. The numbers represent the average of 5 rats per data point.

TABLE W

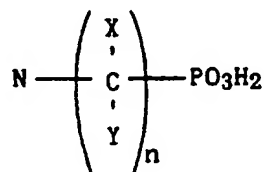
% INJECTED DOSE IN SEVERAL TISSUES FOR Y-EDTMP <sup>1</sup>	
Tissue	% Dose
Bone	30
Liver	0.09
Kidney	0.30
Spleen	0.01
Muscle	0.58
Blood	0.15

<sup>1</sup> Ligand to Y molar ratio of approximately 123

(There are no Examples X and Y.)

#### Example Z (Comparative)

In a method similar to that previously used, compositions were prepared containing complexes of Sm-153 with several commercially available phosphonic acids which do not contain the alkylene linkage between the nitrogen and the phosphorus atoms (which linkage is required in the present ligand).



The two hour biolocalization of Sm-153 in rats for these compositions was determined as previously described. The Ligands used include methylenediphosphonic acid (MDP) and hydroxyethylenediphosphonic acid (HEDP) which contain a P-CH<sub>2</sub>-PO<sub>3</sub>H<sub>2</sub> and a P-C(CH<sub>3</sub>)(OH)-PO<sub>3</sub>H<sub>2</sub> linkage, respectively; pyrophosphate (PYP) which contains a P-O-PO<sub>3</sub>H<sub>2</sub> linkage; and imidodiphosphate (IDP) which contains a N-PO<sub>3</sub>H<sub>2</sub> linkage. Metal complexes of these ligands are known skeletal agents. For example, Tc complexes of MDP, HEDP, and PYP have been used commercially as diagnostic bone agents. However, these ligands were inadequate for selectively delivering Sm-153 to the skeletal system as exemplified by the large fraction of the radioactivity found in the liver and/or blood.

Table Z shows the biolocalization of Sm-153 in rats two hours after injection and the results represent the percent of injected dose in tissue.

TABLE Z

% Dose In	Sm-153 MDP	Sm-153 HEDP	Sm-153 PYP	Sm-153 IDP
Bone	2	21	2	0.6
Liver	85	3.5	73	36
Blood	0.23	13	0.23	0.04

The numbers given in Table Z for Sm-153-MDP, Sm-153-HEDP, Sm-153-PYP and Sm-153-IDP represent the average of the results of five, five, three and three rats, respectively.

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## Example 7: Preparation of Sm-DOTMP or Ho-DOTMP Kit Using HEPES Buffer

A 0.1M solution of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma™ Chemical Co., St. Louis, MO) at a pH of 7.43 was prepared. A 0.0066M solution of DOTMP was prepared by dissolving 68.2 mg ( $1.084 \times 10^{-4}$  mole) of DOTMP in 16.4285 mL of 1N NaOH. Into each of seven 10 mL serum vials was placed 0.600 mL (3.96 mole) of DOTMP solution and 3.00 mL of 0.1M HEPES buffer solution. Each serum vial was then placed in a dry ice/acetone bath until the liquid was frozen and then placed in a Virtis Freeze Dryer Apparatus overnight which gave the aqueous components as a dry white powder in the bottom of the serum vials. The serum vials were then stoppered and sealed by crimping. These kits were formulated to receive 6 mL of either  $\text{SmCl}_3$  ( $3 \times 10^{-4}$  mole) or  $\text{HoCl}_3$  ( $6 \times 10^{-4}$  mole) in 0.1N HCl.

## Example 8: Reconstitution of Sm-DOTMP or Ho-DOTMP Kit Containing HEPES Buffer

A 6.0 mL addition of  $\text{SmCl}_3$  ( $3 \times 10^{-4}$  M spiked with Sm-153 in 0.1N HCl) was made to one of the kits described in Example 7. The pH of the resulting reconstituted kit was 7.5 and the percent of Sm that was complexed was determined using cation exchange chromatography to be >99 percent.

Similarly, a 6.0 mL addition of  $\text{HoCl}_3$  ( $6 \times 10^{-4}$  M spiked with Ho-166) in 0.1N HCl was made to one of the kits described in Example 7. The pH of the resulting solution was 7.5 and the percent of Ho that was complexed was determined using cation exchange chromatography to be >97 percent.

## Example 9: Reconstitution and Biodistribution of Sm-HEPES-DOTMP Kits

A kit from Example 8 was treated with 6.0 mL of  $\text{SmCl}_3$  ( $3 \times 10^{-4}$  M spiked with Sm-153) in 0.1N HCl. The pH of the resulting solution was 7.5 and the percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100  $\mu\text{L}$  of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100  $\mu\text{L}$  standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VII. The numbers represent the average of 3 rats per data point.

TABLE VII

% INJECTED DOSE IN SEVERAL TISSUES FOR Sm-DOTMP/HEPES BUFFER	
Tissue	% Dose
Bone	58
Liver	0.06
Kidney	0.29
Spleen	0.01
Muscle	0.18
Blood	0.06

## Example 10: Preparation of Sm-DOTMP Kits Using Bicarbonate Buffer

A 0.009M solution of DOTMP at pH 6.66 was prepared by adding 141.5 mg ( $2.25 \times 10^{-4}$  mole) of DOTMP to 9 mL of 1N NaOH and diluting to 25 mL final volume. A 0.4M solution of sodium bicarbonate ( $\text{NaHCO}_3$ ) was prepared by dissolving 8.4 g of  $\text{NaHCO}_3$  in 250 mL of water. Kits were prepared by adding 3.0 mL of  $\text{NaHCO}_3$  solution and 0.300 mL of DOTMP solution to each of seven 10 mL serum vials and treating them as described in Example 7 to give the final kit containing a white dry solid. These kits were formulated to receive 6.0 mL of  $\text{SmCl}_3$  ( $3 \times 10^{-4}$  M) in 0.1N HCl which would give a ligand to metal ratio of

1.5:1.

**Example 11: Reconstitution and Biodistribution of Sm-DOTMP Kits Using Bicarbonate Buffer**

5 A kit from Example 10 was treated with 6.0 mL of  $\text{SmCl}_3$  ( $3 \times 10^{-4}\text{M}$  spiked with Sm-153) in 0.1N HCl. The pH of the resulting solution was 6.55 and was adjusted to 7.27 by the addition of 60  $\mu\text{L}$  of 1N NaOH. The percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

10 Sprague Dawley rats were allowed to acclimate for five days then injected with 100  $\mu\text{L}$  of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100  $\mu\text{L}$  standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose  
15 in several tissues are given in Table VIII. The numbers represent the average of 3 rats per data point.

TABLE VIII

	% INJECTED DOSE IN SEVERAL TISSUES FOR Sm-DOTMP <sup>1</sup> /BICARBONATE	
20	Tissue	% Dose
	Bone	65
	Liver	0.07
25	Kidney	0.34
	Spleen	0.01
	Muscle	0.30
30	Blood	0.04

<sup>1</sup> Ligand to Sm molar ratio of approximately 1.5

**Example 12: Preparation of DOTMP Kit Using Excess Base**

35 A 0.009M solution of DOTMP was prepared as described in Example 10 except more NaOH was added such that the final solution was pH 10.66. Kits were prepared by adding 0.300 mL of DOTMP solution and 0.700 mL of 1.0N NaOH solution to each of five 10 mL serum vials and treating them as described in  
40 Example 7 to give the final kit containing a white dry solid. These kits were formulated to receive 6.0 mL of  $\text{SmCl}_3$  ( $3 \times 10^{-4}\text{M}$ ) in 0.1N HCl which would give a ligand to metal ratio of 1.5:1.

**Example 13: Reconstitution and Biodistribution of DOTMP Kits Using Excess Base and Phosphate Buffer**

45 A kit from Example 12 was treated with 5.4 mL of  $\text{SmCl}_3$  ( $3 \times 10^{-4}\text{M}$  spiked with Sm-153) in 0.1N HCl and 0.6 mL of  $\text{SmCl}_3$  ( $3 \times 10^{-4}\text{M}$  spiked with Sm-153) in 0.1N HCl. The pH of the resulting solution was between 10 and 11. The pH was adjusted to 7.79 by the addition of 0.200 mL of 1.05M phosphate buffer (pH 7.49). The percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

50 Sprague Dawley rats were allowed to acclimate for five days then injected with 100  $\mu\text{L}$  of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100  $\mu\text{L}$  standards in order  
55 to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table IX. The numbers represent the average of 5 rats per data point.



TABLE IX

% INJECTED DOSE IN SEVERAL TISSUES FOR Sm-DOTMP <sup>1</sup> /PHOSPHATE	
Tissue	% Dose
Bone	59
Liver	0.85
Kidney	0.41
Spleen	0.03
Muscle	0.35
Blood	0.11

<sup>1</sup> Ligand to Sm molar ratio of approximately 1.5

#### Example 14: Preparation of 18 mL Ho-DOTMP Kits

A 0.009M solution of DOTMP at pH 6.66 was prepared as described in Example 10 except more NaOH was added such that the final solution was at pH 10.19. Kits were prepared by adding 1.800 mL of DOTMP solution and 2.100 mL of 1N NaOH solution to each of twelve 20 mL serum vials. These vials were then treated as described in Example 7 to give the final kits containing a white, dry solid. These kits were formulated to receive 18.0 mL of HoCl<sub>3</sub> ( $6 \times 10^{-4}$  M) which would give a ligand to metal ratio of 1.5:1.

#### Example 15: Reconstitution and Biodistribution of 18 mL Ho-DOTMP Kits

A kit from Example 14 was treated with 18.0 mL of HoCl<sub>3</sub> ( $6 \times 10^{-4}$  M spiked with Ho-166) in 0.1N HCl. The solution was then treated with 0.6 mL of 1.05M phosphate buffer (pH 7.49) which brought the pH down to 7.53. The percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100  $\mu$ L of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100  $\mu$ L standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table X. The numbers represent the average of 5 rats per data point.

TABLE X

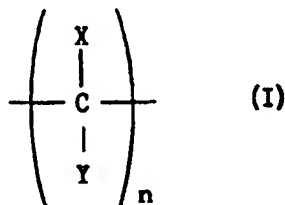
% INJECTED DOSE IN SEVERAL TISSUES FOR Ho-DOTMP <sup>1</sup> /PHOSPHATE	
Tissue	% Dose
Bone	60
Liver	0.12
Kidney	0.35
Spleen	0.08
Muscle	0.21
Blood	0.04

<sup>1</sup> Ligand to Ho molar ratio of approximately 1.5

## Claims

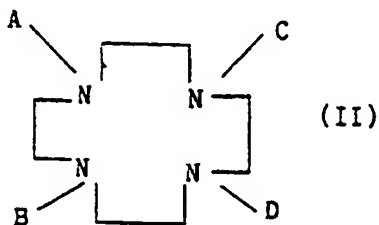
Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorous are interconnected by an alkylene or substituted alkylene radical of the formula

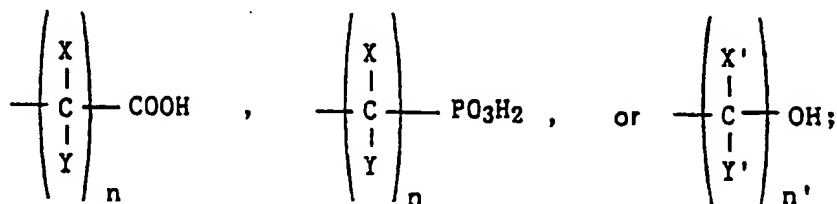


wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the proviso that when n>1, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, and wherein the resulting composition is therapeutically effective.

2. The composition of Claim 1 wherein X and Y are hydrogen and n is 1.
3. The composition of Claim 1 or Claim 2, wherein the macrocyclic aminophosphonic acid has the structure



wherein: substituents A, B, C and D are independently hydrogen, a hydrocarbon radical having from 1-8 carbon atoms, or a moiety of the formula



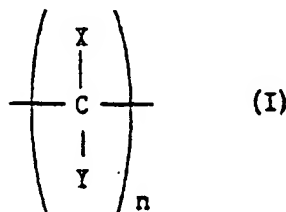
or a physiologically acceptable salt of such an acid radical, wherein: X, Y and n are as defined in Claim 1 or Claim 2; X' and Y' are independently hydrogen, methyl or ethyl; and n' is 2 or 3, with the proviso that at least two of said nitrogen substituents is a phosphorus-containing group.

4. The composition of Claim 3 wherein the macrocyclic aminophosphonic acid is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt.
5. The composition of any one of the preceding claims wherein the radionuclide is Gd-159.
- 5 6. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Sm-153.
7. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Lu-177.
- 10 8. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Yb-175.
9. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Ho-166.
10. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Y-90.
- 15 11. A sterile composition in dosage form suitable for administration to an animal wherein the composition contains a complex as claimed in any one of the preceding claims and wherein the radionuclide is present in the composition dose in an amount containing at least 0.02 mCi per kilogram of body weight of said animal.
- 20 12. The composition of Claim 11 wherein the radionuclide in dosage form is present in an amount containing at least 0.2 mCi per kilogram of body weight of said animal.
13. The composition of any one of the preceding claims wherein the ligand to radionuclide molar ratio is at least 1:1.
- 25 14. The composition of Claim 13 wherein the ligand to radionuclide molar ratio is from 1:1 to 3:1.
15. The composition of Claim 13 wherein the ligand to radionuclide molar ratio is from 1:1 to 1.5:1.
- 30 16. A pharmaceutical formulation which comprises the composition as claimed in any one of Claims 1 to 10, together with a pharmaceutically acceptable carrier.
17. A pharmaceutical formulation of Claim 16 wherein the formulation having the complex and a buffer present is frozen in a kit form, and which frozen formulation is later thawed prior to use.
- 35 18. The use of a composition as claimed in Claim 1 in the preparation of a medicament for the therapeutic treatment of an animal having one or more calcific tumor or bone pain.
- 40 19. A process for preparing a composition as claimed in Claim 1, which comprises reacting a radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175 with the macrocyclic aminophosphonic acid as defined in any one of the preceding claims, in water at a controlled pH.
- 45 20. The process of Claim 19 which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Sm-153, in water at a controlled pH.
21. The process of Claim 19 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Gd-159, in water at a controlled pH.
- 50 22. The process of Claim 19 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Ho-166, in water at a controlled pH.
- 55 23. The process of Claim 19 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Lu-177, in water at a controlled pH.

24. The process of Claim 19 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Yb-175, in water at a controlled pH.
- 5 25. The process of Claim 19 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Y-90, in water at a controlled pH.
- 10 26. A kit for the production of a composition as claimed in any one of Claims 1 to 10, comprising means for containing separately a macrocyclic aminophosphonic acid complex as defined in Claim 1 and a radionuclide as defined in Claim 1, such that the said macrocyclic aminophosphonic acid and radionuclide may be mixed prior to use to form the said composition.

**Claims for the following Contracting State : ES**

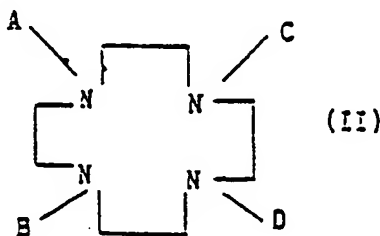
- 15 1. A process for preparing a therapeutically effective composition which process comprises reacting (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorous are interconnected by an alkylene or substituted alkylene radical of the formula



wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the proviso that when n>1, each X and Y may be the same as or different from the X and Y of any other carbon atom, with (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175.

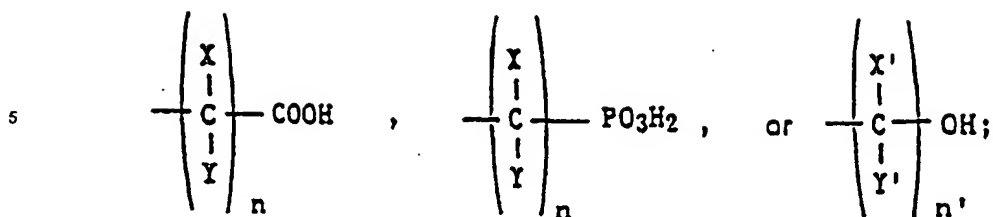
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2. The process of Claim 1 wherein X and Y are hydrogen and n is 1.
- 40 3. The process of Claim 1 or Claim 2, wherein the macrocyclic aminophosphonic acid has the structure



wherein: substituents A, B, C and D are independently hydrogen, a hydrocarbon radical having from 1-8 carbon atoms, or a moiety of the formula

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or a physiologically acceptable salt of such an acid radical, wherein: X, Y and n are as defined in Claim 1 or Claim 2; X' and Y' are independently hydrogen, methyl or ethyl; and n' is 2 or 3, with the proviso that at least two of said nitrogen substituents is a phosphorus-containing group.

- 15 4. The process of Claim 3 wherein the macrocyclic aminophosphonic acid is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt.
5. The process of any one of the preceding claims wherein the radionuclide is Gd-159.
- 20 6. The process of Claim 1, 2, 3 or 4 wherein the radionuclide is Sm-153.
7. The process of Claim 1, 2, 3 or 4 wherein the radionuclide is Lu-177.
8. The process of Claim 1, 2, 3 or 4 wherein the radionuclide is Yb-175.
- 25 9. The process of Claim 1, 2, 3 or 4 wherein the radionuclide is Ho-166.
10. The process of Claims 1, 2, 3 or 4 wherein the radionuclide is Y-90.
- 30 11. The process of any one of the preceding claims, and including the step of formulating the composition in dosage form suitable for administration to an animal, wherein the radionuclide is present in the composition dose in an amount containing at least 0.02 mCi per kilogram of body weight of said animal.
12. The process of Claim 11 wherein the radionuclide in dosage form is present in an amount containing at least 0.2 mCi per kilogram of body weight of said animal.
- 35 13. The process of any one of the preceding claims wherein the ligand to radionuclide molar ratio is at least 1:1.
- 40 14. The process of Claim 13 wherein the ligand to radionuclide molar ratio is from 1:1 to 3:1.
15. The process of Claim 13 wherein the ligand to radionuclide molar ratio is from 1:1 to 1.5:1.
16. A process as claimed in any one of the preceding claims, wherein the composition also comprises a pharmaceutically acceptable carrier.
- 45 17. A process as claimed in any one of the preceding claims, and including the steps of incorporating a buffer into the composition, and freezing the composition in a kit form.
- 50 18. A process as claimed in any one of the preceding claims, wherein the reaction is carried out in water at a controlled pH.
19. The process of Claim 18 which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Sm-153, in water at a controlled pH.
- 55 20. The process of Claim 18, which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Gd-159, in water at a controlled

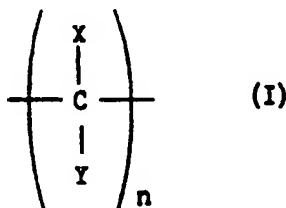
pH.

21. The process of Claim 18, which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Ho-166, in water at a controlled pH.
22. The process of Claim 18, which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Lu-177, in water at a controlled pH.
23. The process of Claim 18, which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Yb-175, in water at a controlled pH.
24. The process of Claim 18, which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Y-90, in water at a controlled pH.
25. A kit for the production of a composition as claimed in any one of Claims 1 to 10, comprising means for containing separately a macrocyclic aminophosphonic acid complex as defined in Claim 1 and a radionuclide as defined in Claim 1, such that the said macrocyclic aminophosphonic acid and radionuclide may be mixed prior to use to form the said composition.

#### Patentansprüche

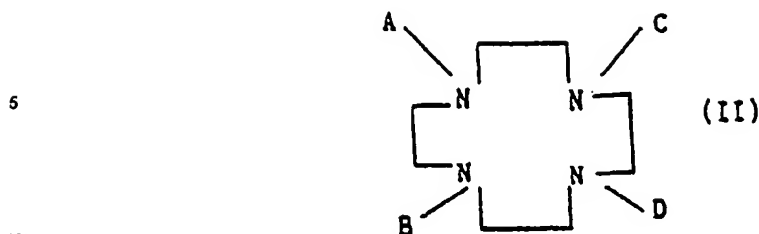
Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Zusammensetzung, welche einen Komplex umfaßt, der aufweist (1) eine makrocyclische, 1,4,7,10-Tetraazacyclodecan als die makrocyclische Einheit enthaltende Aminophosphonsäure oder ein physiologisch annehmbares Salz hiervon, worin der Stickstoff und Phosphor miteinander verbunden sind durch einen Alkyl- oder substituierten Alkylrest der Formel:



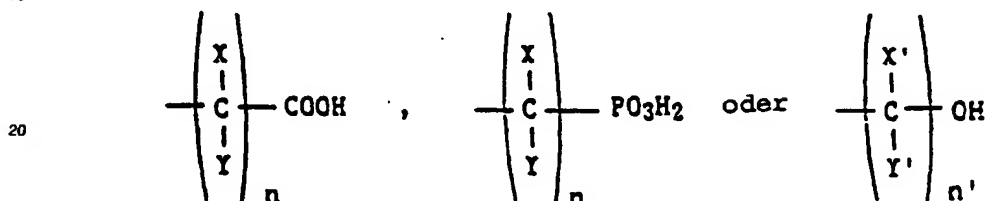
worin: X und Y unabhängig voneinander Wasserstoff-, Hydroxyl-, Carboxyl-, Phosphon- oder Kohlenwasserstoffreste mit 1 bis 18 Kohlenstoffatomen und physiologisch annehmbare Salze der Säurereste sind und  $n = 1 - 3$  ist, mit der Maßgabe daß, falls  $n > 1$ , jedes X und Y gleich oder verschieden sein kann von dem X und Y irgendeines anderen Kohlenstoffatoms, und (2) wenigstens ein Radionuklid von Sm-153, Gd-159, Ho-166, Lu-177, Y-90 oder Yb-175, und wobei die erhaltene Zusammensetzung therapeutisch wirksam ist.

2. Zusammensetzung nach Anspruch 1, worin X und Y Wasserstoff sind und  $n = 1$  ist.
3. Zusammensetzung nach Anspruch 1 oder Anspruch 2, worin die makrocyclische Aminophosphonsäure die Struktur besitzt:



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worin die Substituenten A, B, C und D unabhängig voneinander sind: Wasserstoff, ein Kohlenwasserstoffrest mit 1 - 8 Kohlenstoffatomen oder eine Einheit der Formel



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oder ein physiologisch annehmbares Salz solch eines Säurerestes, worin: X, Y und n wie in Anspruch 1 oder Anspruch 2 definiert sind; X' und Y' unabhängig voneinander Wasserstoff, Methyl oder Ethyl sind und n' = 2 oder 3 ist, mit der Maßgabe, daß wenigstens zwei dieser Stickstoffsubstituenten eine phosphorhaltige Gruppe sind.

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4. Zusammensetzung nach Anspruch 3, worin die makrocyclische Aminophosphonsäure 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder ein physiologisch annehmbares Salz hiervon ist.

5. Zusammensetzung nach einem der vorhergehenden Ansprüche, worin das Radionuklid Gd-159 ist.

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6. Zusammensetzung nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Sm-153 ist.

7. Zusammensetzung nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Lu-177 ist.

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8. Zusammensetzung nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Yb-175 ist.

9. Zusammensetzung nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Ho-166 ist.

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10. Zusammensetzung nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Y-90 ist.

11. Sterile Zusammensetzung in für die Applikation bei einem Tier geeigneter Dosierungsform, worin die Zusammensetzung einen Komplex, wie in einem der vorhergehenden Ansprüche beansprucht, enthält und worin das Radionuklid in der Zusammensetzungsform in einer Menge vorhanden ist, welche wenigstens 0,02 mCi pro Kilogramm Körpergewicht dieses Tieres enthält.

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12. Zusammensetzung nach Anspruch 11, worin das Radionuklid in Dosierungsform in einer Menge vorhanden ist, welche wenigstens 0,2 mCi pro Kilogramm Körpergewicht dieses Tieres enthält.

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13. Zusammensetzung nach einem der vorhergehenden Ansprüche, worin das Molverhältnis Ligand-zu-Radionuklid wenigstens 1 : 1 ist.

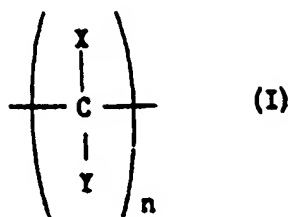
14. Zusammensetzung nach Anspruch 13, worin das Molverhältnis Ligand-zu-Radionuklid von 1 : 1 bis 3 : 1 ist.

15. Zusammensetzung nach Anspruch 13, worin das Molverhältnis Ligand-zu-Radionuklid von 1 : 1 bis 1,5 : 1 ist.
16. Pharmazeutische Formulierung, welche die Zusammensetzung, wie in einem der Ansprüche 1 bis 10 beansprucht, zusammen mit einem pharmazeutisch annehmbaren Träger umfaßt.
17. Pharmazeutische Formulierung nach Anspruch 16, worin die Formulierung, welche den Komplex und einen vorhandenen Puffer aufweist, in einer gefrorenen Kit-Form vorliegt, wobei diese gefrorene Formulierung später vor der Anwendung aufgetaut wird.
18. Verwendung einer Zusammensetzung, wie in Anspruch 1 beansprucht, bei der Herstellung eines Arzneimittels für die therapeutische Behandlung eines Tieres, das einen oder mehrere kalkbildende Tumore oder Knochenschmerzen hat.
19. Verfahren zur Herstellung einer Zusammensetzung, wie in Anspruch 1 beansprucht, welches umfaßt: die Umsetzung eines Radionuklids von Sm-153, Gd-159, Ho-166, Lu-177, Y-90 oder Yb-175 mit der makrocyclischen Aminophosphonsäure, wie in einem der vorhergehenden Ansprüche definiert, in Wasser bei einem kontrollierten pH.
20. Verfahren nach Anspruch 19, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Sm-153 in Wasser bei einem kontrollierten pH umfaßt.
21. Verfahren nach Anspruch 19 zur Herstellung einer Zusammensetzung, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Gd-159 in Wasser bei einem kontrollierten pH umfaßt.
22. Verfahren nach Anspruch 19 zur Herstellung einer Zusammensetzung, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Ho-166 in Wasser bei einem kontrollierten pH umfaßt.
23. Verfahren nach Anspruch 19 zur Herstellung einer Zusammensetzung, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Lu-177 in Wasser bei einem kontrollierten pH umfaßt.
24. Verfahren nach Anspruch 19 zur Herstellung einer Zusammensetzung, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Yb-175 in Wasser bei einem kontrollierten pH umfaßt.
25. Verfahren nach Anspruch 19 zur Herstellung einer Zusammensetzung, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Y-90 in Wasser bei einem kontrollierten pH umfaßt.
26. Kit zur Herstellung einer Zusammensetzung, wie in einem der Ansprüche 1 bis 10 beansprucht, umfassend Mittel zur getrennten Aufbewahrung eines makrocyclischen Aminophosphonsäure-Komplexes, wie in Anspruch 1 definiert, und eines Radionuklids, wie in Anspruch 1 definiert, derart, daß diese makrocyclische Aminophosphonsäure und dieses Radionuklid vor der Anwendung zur Bildung dieser Zusammensetzung vermischt werden können.

**Patentansprüche für folgenden Vertragsstaat : ES**

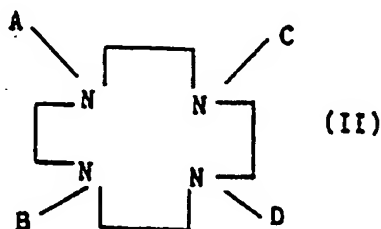
1. Verfahren zur Herstellung einer therapeutisch wirksamen Zusammensetzung, wobei das Verfahren die Umsetzung umfaßt von (1) einer makrocyclischen, 1,4,7,10-Tetraazacyclodecan als die makrocyclische Einheit enthaltenden Aminophosphonsäure oder eines physiologisch annehmbaren Salz hiervon, worin der Stickstoff und Phosphor miteinander verbunden sind durch einen Alkylen- oder substituierten Alkylenrest der Formel:



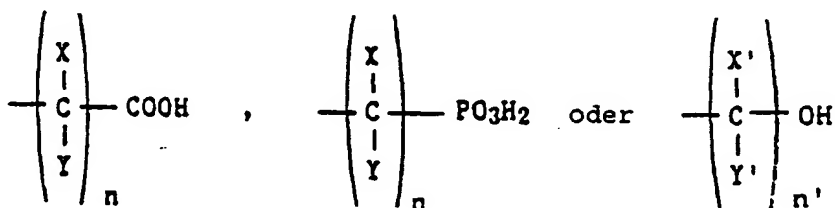


worin: X und Y unabhängig voneinander Wasserstoff-, Hydroxyl-, Carboxyl-, Phosphon- oder Kohlenwasserstoffreste mit 1 bis 18 Kohlenstoffatomen und physiologisch annehmbare Salze der Säurereste sind und  $n = 1 - 3$  ist, mit der Maßgabe daß, falls  $n > 1$ , Jedes X und Y gleich oder verschieden sein kann von dem X und Y irgendeines anderen Kohlenstoffatoms, mit (2) wenigstens einem Radionuklid von Sm-153, Gd-159, Ho-166, Lu-177, Y-90 oder Yb-175.

2. Verfahren nach Anspruch 1, worin X und Y Wasserstoff sind und  $n = 1$  ist.
3. Verfahren nach Anspruch 1 oder Anspruch 2, worin die makrocyclische Aminophosphonsäure die Struktur besitzt:



worin die Substituenten A, B, C und D unabhängig voneinander sind: Wasserstoff, ein Kohlenwasserstoffrest mit 1 - 8 Kohlenstoffatomen oder eine Einheit der Formel



oder ein physiologisch annehmbares Salz solch eines Säurerestes, worin: X, Y und  $n$  wie in Anspruch 1 oder Anspruch 2 definiert sind; X' und Y' unabhängig voneinander Wasserstoff, Methyl oder Ethyl sind und  $n' = 2$  oder 3 ist, mit der Maßgabe, daß wenigstens zwei dieser Stickstoffsubstituenten eine phosphorhaltige Gruppe sind.

4. Verfahren nach Anspruch 3, worin die makrocyclische Aminophosphonsäure 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder ein physiologisch annehmbares Salz hiervon ist.
5. Verfahren nach einem der vorhergehenden Ansprüche, worin das Radionuklid Gd-159 ist.
6. Verfahren nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Sm-153 ist.
7. Verfahren nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Lu-177 ist.

8. Verfahren nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Yb-175 ist.
9. Verfahren nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Ho-166 ist.
- 5 10. Verfahren nach Anspruch 1, 2, 3 oder 4, worin das Radionuklid Y-90 ist.
11. Verfahren nach einem der vorhergehenden Ansprüche und einschließlich die Stufe der Formulierung der Zusammensetzung in für die Applikation bei einem Tier geeignete Dosierungsform, worin das Radionuklid in der Zusammensetzungs-dosis in einer Menge vorhanden ist, welche wenigstens 0,02 mCi  
10 pro Kilogramm Körpergewicht dieses Tieres enthält.
12. Verfahren nach Anspruch 11, worin das Radionuklid in Dosierungsform in einer Menge vorhanden ist, welche wenigstens 0,2 mCi pro Kilogramm Körpergewicht dieses Tieres enthält.
- 15 13. Verfahren nach einem der vorhergehenden Ansprüche, worin das Molverhältnis Ligand-zu-Radionuklid wenigstens 1 : 1 ist.
14. Verfahren nach Anspruch 13, worin das Molverhältnis Ligand-zu-Radionuklid von 1 : 1 bis 3 : 1 ist.
- 20 15. Verfahren nach Anspruch 13, worin das Molverhältnis Ligand-zu-Radionuklid von 1 : 1 bis 1,5 : 1 ist.
16. Verfahren nach einem der vorhergehenden Ansprüche, worin die Zusammensetzung weiterhin einem pharmazeutisch annehmbaren Träger umfaßt.
- 25 17. Verfahren nach einem der vorhergehenden Ansprüche und einschließlich die Stufen der Eingabe eines Puffers in die Zusammensetzung und Einfrieren der Zusammensetzung in einer Kit-Form.
18. Verfahren nach einem der vorhergehenden Ansprüche, worin die Reaktion in Wasser bei einem kontrollierten pH durchgeführt wird.
- 30 19. Verfahren nach Anspruch 18, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Sm-153 in Wasser bei einem kontrollierten pH umfaßt.
- 35 20. Verfahren nach Anspruch 18, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Gd-159 in Wasser bei einem kontrollierten pH umfaßt.
- 40 21. Verfahren nach Anspruch 18, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Ho-166 in Wasser bei einem kontrollierten pH umfaßt.
- 45 22. Verfahren nach Anspruch 18, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Lu-177 in Wasser bei einem kontrollierten pH umfaßt.
- 50 23. Verfahren nach Anspruch 18, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Yb-175 in Wasser bei einem kontrollierten pH umfaßt.
- 55 24. Verfahren nach Anspruch 18, welches die Umsetzung von 1,4,7,10-Tetraazacyclodecan-1,4,7,10-tetramethylenphosphonsäure oder eines physiologisch annehmbaren Salzes mit Y-90 in Wasser bei einem kontrollierten pH umfaßt.
25. Kit zur Herstellung einer Zusammensetzung, wie in einem der Ansprüche 1 bis 10 beansprucht, umfassend Mittel zur getrennten Aufbewahrung eines makrocyclischen Aminophosphonsäure-Komplexes, wie in Anspruch 1 definiert, und eines Radionuklids, wie in Anspruch 1 definiert, derart, daß die makrocyclische Aminophosphonsäure und dieses Radionuklid vor der Anwendung zur Bildung dieser

Zusammensetzung vermischt werden können.

# Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

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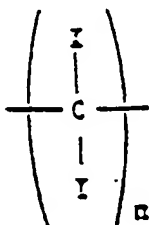
1. Composition qui comprend un complexe, qui contient:

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a) un acide aminophosphonique macrocyclique, comportant un cycle 1,4,7,10-tétraazacyclododécane en tant que fragment macrocyclique, ou un sel physiologiquement acceptable d'un tel acide, les atomes d'azote et de phosphore étant reliés par des radicaux alkylène, substitués ou non, de formule

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(I)

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dans laquelle X et Y représentent chacun, indépendamment, un atome d'hydrogène, un groupe hydroxyle, carboxyle ou phosphonique, un radical hydrocarboné comportant de 1 à 8 atomes de carbone, ou un groupe acide salifié de façon physiologiquement acceptable, et n vaut de 1 à 3, chaque X et chaque Y pouvant, quand n est supérieur à 1, être identiques aux X et Y liés à n'importe quel autre atome de carbone ou différents de ces X et Y, et

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b) au moins un radionucléide choisi parmi Sm-153, Gd-159, Ho-166, Lu-177, Y-90 et Yb-175, la composition résultante présentant une efficacité thérapeutique.

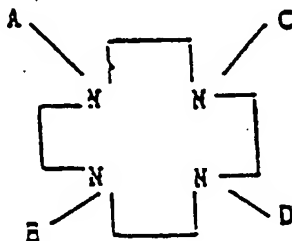
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2. Composition conforme à la revendication 1, dans laquelle X et Y représentent des atomes d'hydrogène et n vaut 1.

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3. Composition conforme à la revendication 1 ou 2, dans laquelle l'acide aminophosphonique macrocyclique présente la structure suivante:

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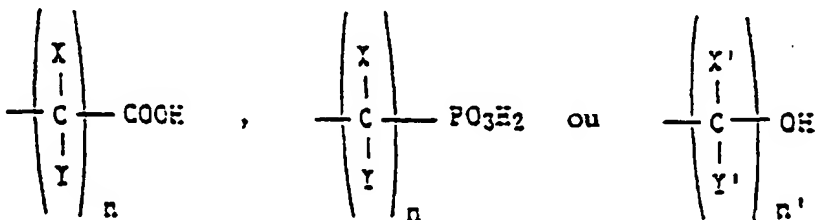


(II)

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dans laquelle les substituants A, B, C et D représentent chacun, indépendamment, un atome d'hydrogène, un radical hydrocarboné comportant de 1 à 8 atomes de carbone, ou un fragment de formule

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ou un tel fragment acid salifié de façon physiologiquement acceptable, X, Y et n ayant les définitions indiquées dans la revendication 1 ou 2, X' et Y' représentant chacun, indépendamment, un atome d'hydrogène ou un groupe méthyle ou éthyle, et n' valant 2 ou 3, sous réserve qu'au moins deux desdits substituants placés sur des atomes d'azote soient des groupes phosphorés.

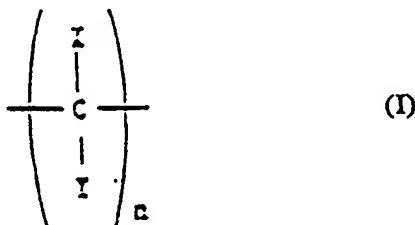
- 5
4. Composition conforme à la revendication 3, dans laquelle l'acide aminophosphonique macrocyclique est l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique ou l'un de ses sels physiologiquement acceptables.
- 10 5. Composition conforme à l'une des revendications précédentes, dans laquelle le radionucléide est Gd-159.
6. Composition conforme à la revendication 1,2,3 ou 4, dans laquelle le radionucléide est Sm-153.
- 15 7. Composition conforme à la revendication 1,2,3 ou 4, dans laquelle le radionucléide est Lu-177.
8. Composition conforme à la revendication 1,2,3 ou 4, dans laquelle le radionucléide est Yb-175.
9. Composition conforme à la revendication 1,2,3 ou 4, dans laquelle le radionucléide est Ho-166.
- 20 10. Composition conforme à la revendication 1, 2, 3 ou 4, dans laquelle le radionucléide est Y-90.
11. Composition stérile sous forme posologique convenable pour être administrée à un animal, la composition contenant un complexe défini dans l'une des revendications précédentes, et le radionucléide se trouvant, dans la dose de composition, en une quantité correspondant à une activité d'au moins 0,02 mCi par kilogramme de poids corporel dudit animal.
- 25 12. Composition conforme à la revendication 11, dans laquelle le radionucléide se trouve, dans la forme posologique, en une quantité correspondant à une activité d'au moins 0,2 mCi par kilogramme de poids corporel dudit animal.
- 30 13. Composition conforme à l'une des revendications précédentes, dans laquelle le rapport molaire du ligand au radionucléide vaut au moins 1/1.
- 35 14. Composition conforme à la revendication 13, dans laquelle le rapport molaire du ligand au radionucléide vaut de 1/1 à 3/1.
15. Composition conforme à la revendication 13, dans laquelle le rapport molaire du ligand au radionucléide vaut de 1/1 à 1,5/1.
- 40 16. Formulation pharmaceutique qui contient une composition conforme à l'une des revendications 1 à 10, conjointement avec un véhicule acceptable en pharmacie.
17. Formulation pharmaceutique conforme à la revendication 16, dans laquelle se trouvent le complexe et un agent tampon et qui est congelée, dans un nécessaire, la formulation congelée étant ultérieurement dégelée avant d'être utilisée.
- 45 18. Emploi d'une composition, conforme à la revendication 1, dans la préparation d'un médicament destiné au traitement thérapeutique d'un animal souffrant d'une ou de plusieurs tumeurs calcifiantes ou douleurs osseuses.
- 50 19. Procédé de préparation d'une composition conforme à la revendication 1, qui comporte le fait de faire réagir un radionucléide, choisi parmi Sm-153, Gd-159, Ho-166, Lu-177, Y-90 et Yb-175, avec un acide aminophosphonique macrocyclique défini dans l'une des revendications précédentes, dans de l'eau et à un pH réglé.
- 55 20. Procédé de préparation d'une composition, conforme à la revendication 19, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un d ses

sels physiologiquement acceptables, avec du Sm-153, dans de l'eau et à un pH réglé.

21. Procédé de préparation d'une composition, conforme à la revendication 19, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Gd-159, dans de l'eau et à un pH réglé.
22. Procédé de préparation d'une composition, conforme à la revendication 19, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Ho-166, dans de l'eau et à un pH réglé.
23. Procédé de préparation d'une composition, conforme à la revendication 19, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Lu-177, dans de l'eau et à un pH réglé.
24. Procédé de préparation d'une composition, conforme à la revendication 19, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Yb-175, dans de l'eau et à un pH réglé.
25. Procédé de préparation d'une composition, conforme à la revendication 19, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Y-90, dans de l'eau et à un pH réglé.
26. Nécessaire pour la production d'une composition conforme à l'une des revendications 1 à 10, comportant des récipients contenant séparément un acide aminophosphonique macrocyclique défini dans la revendication 1 et un radionucléide défini dans la revendication 1, de façon que l'on puisse mélanger l'acide aminophosphonique macrocyclique et le radionucléide avant l'emploi, pour former ladite composition.

#### Revendications pour l'Etat contractant suivant : ES

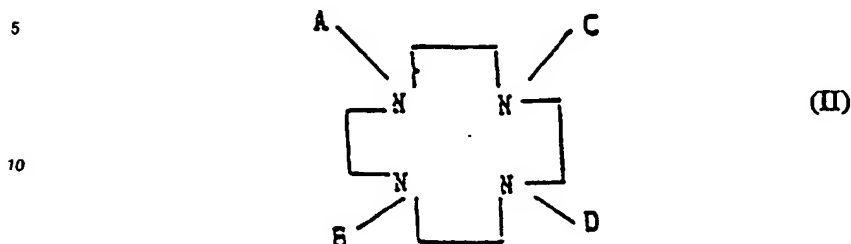
1. Procédé de préparation d'une composition qui possède une activité thérapeutique, lequel procédé comprend le fait de faire réagir
  - a) un acide aminophosphonique macrocyclique, comportant un cycle 1,4,7,10-tétraazacyclododécane en tant que fragment macrocyclique, ou un sel physiologiquement acceptable d'un tel acide, les atomes d'azote et de phosphore étant reliés par des radicaux alkylène, substitués ou non, de formule



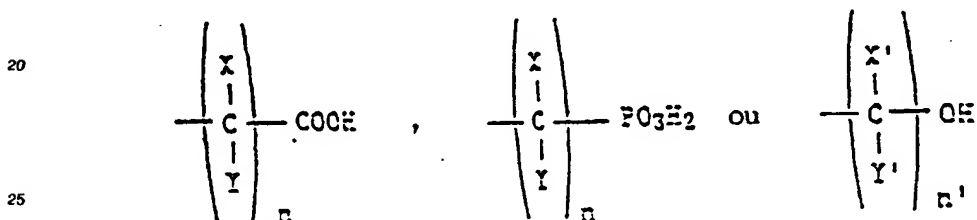
- dans laquelle X et Y représentent chacun, indépendamment, un atome d'hydrogène, un groupe hydroxyle, carboxyle ou phosphonique, un radical hydrocarboné comportant de 1 à 8 atomes de carbone, ou un groupe acide salifié de façon physiologiquement acceptable, et n vaut de 1 à 3, chaque X et chaque Y pouvant, quand n est supérieur à 1, être identiques aux X et Y liés à n'importe quel autre atome de carbone ou différents de ces X et Y, avec
- b) au moins un radionucléide choisi parmi Sm-153, Gd-159, Ho-166, Lu-177, Y-90 et Yb-175, la composition résultante présentant une efficacité thérapeutique.

2. Procédé conforme à la revendication 1, dans lequel X et Y représentent des atomes d'hydrogène et n vaut 1.

3. Procédé conforme à la revendication 1 ou 2, dans lequel l'acide aminophosphonique macrocyclique présente la structure :



- 15 dans laquelle les substituants A, B, C et D représentent chacun, indépendamment, un atome d'hydrogène, un radical hydrocarboné comportant de 1 à 8 atomes de carbone, ou un fragment de formule



- 30 ou un tel fragment acide salifié de façon physiologiquement acceptable, X, Y et n ayant les définitions indiquées dans la revendication 1 ou 2, X' et Y' représentant chacun, indépendamment, un atome d'hydrogène ou un groupe méthyle ou éthyle, et n' valant 2 ou 3, sous réserve qu'au moins deux desdits substituants placés sur des atomes d'azote soient des groupes phosphorés.

- 35 4. Procédé conforme à la revendication 3, dans lequel l'acide aminophosphonique macrocyclique est l'acide 1,4,7,10-tétra-azacyclo-dodéane-1,4,7,10-tétraméthylène-phosphonique ou l'un de ses sels physiologiquement acceptables.
- 40 5. Procédé conforme à l'une des revendications précédentes, dans lequel le radionucléide est Gd-159.
6. Procédé conforme à la revendication 1, 2, 3 ou 4, dans lequel le radionucléide est Sm-153.
7. Procédé conforme à la revendication 1, 2, 3 ou 4, dans lequel le radionucléide est Lu-177.
8. Procédé conforme à la revendication 1, 2, 3 ou 4, dans lequel le radionucléide est Yb-175.
- 45 9. Procédé conforme à la revendication 1, 2, 3 ou 4, dans lequel le radionucléide est Ho-166.
10. Procédé conforme à la revendication 1,2,3 ou 4, dans lequel le radionucléide est Y-90.
- 50 11. Procédé conforme à l'une des revendications précédentes, comprenant une étape consistant à formuler la composition sous une forme posologique convenable pour être administrée à un animal, le radionucléide se trouvant, dans la dose de composition, en une quantité correspondant à une activité d'au moins 0,02 mCi par kilogramme de poids corporel dudit animal.
- 55 12. Procédé conforme à la revendication 11, dans lequel le radionucléide se trouve, dans la forme posologique, en une quantité correspondant à une activité d'au moins 0,2 mCi par kilogramme de poids corporel dudit animal.

13. Procédé conforme à l'une des revendications précédentes, dans lequel le rapport molaire du ligand au radionucléide vaut au moins 1/1.
- 5 14. Procédé conforme à la revendication 13, dans lequel le rapport molaire du ligand au radionucléide vaut de 1/1 à 3/1.
- 15 15. Procédé conforme à la revendication 13, dans lequel le rapport molaire du ligand au radionucléide vaut de 1/1 à 1,5/1.
- 10 16. Procédé conforme à l'une des revendications précédentes, dans lequel la composition comprend aussi un véhicule acceptable en pharmacie.
- 15 17. Procédé conforme à l'une des revendications précédentes, qui comporte les étapes consistant à incorporer un agent tampon dans la composition et à congeler la composition en la mettant sous forme d'un nécessaire.
18. Procédé conforme à l'une des revendications précédentes, dans lequel on effectue la réaction dans de l'eau et à un pH réglé.
- 20 19. Procédé conforme à la revendication 18, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Sm-153, dans de l'eau et à un pH réglé.
- 25 20. Procédé conforme à la revendication 18, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Gd-159, dans de l'eau et à un pH réglé.
- 30 21. Procédé conforme à la revendication 18, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Ho-166, dans de l'eau et à un pH réglé.
- 35 22. Procédé conforme à la revendication 18, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Lu-177, dans de l'eau et à un pH réglé.
- 40 23. Procédé conforme à la revendication 18, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Yb-175, dans de l'eau et à un pH réglé.
- 45 24. Procédé conforme à la revendication 18, qui comporte le fait de faire réagir de l'acide 1,4,7,10-tétraazacyclododécane-1,4,7,10-tétraméthylène phosphonique, ou l'un de ses sels physiologiquement acceptables, avec du Y-90, dans de l'eau et à un pH réglé.
- 50 25. Nécessaire pour la production d'une composition préparée conformément à l'une des revendications 1 à 10, comportant des récipients contenant séparément un acide aminophosphonique macrocyclique défini dans la revendication 1 et un radionucléide défini dans la revendication 1, de façon que l'on puisse mélanger l'acide aminophosphonique macrocyclique et le radionucléide avant l'emploi, pour former ladite composition.

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